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In silico investigation of the antiemetic effect of cannabidiolic acid (CBDA), the phytocannabinoid of Cannabis sativa, by molecular docking method

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Abstract

Aim: *Cannabis sativa* has essential secondary metabolites in the treatment of diseases. The effects of the phytocannabinoids of *Cannabis sativa* on emesis continue to be investigated. In this study, we aimed to investigate the antiemetic effect of cannabidiol acid, one of the phytocannabinoids of the *Cannabis sativa* plant, by in silico methods.

Material and Methods: In this study, the molecular properties of cannabidiolic acid obtained from the PubChem database were investigated using the SwissADME database. To examine the effects of cannabidiolic acid on vomiting, the serotonin receptor was determined as the target protein. The target protein was obtained from the protein database, and molecular insertion was performed using AutoDock Vina. The docking process was visualized using Discovery Studio Visualizer and LigPlot imaging programs.

Results: According to our clamping study, the excellent hydrogen and hydrophobic interactions shown by CBDA on the 5HT3A receptor appear to be more effective than the reference drugs used.

Discussion: The examination found that cannabidiolic acid complies with Lipinski's rules and has better binding energy, so it is a suitable candidate for the treatment of emesis.

Keywords

In-Silico, Cannabidiolic Acid (CBDA), Molecular Docking, Emesis, Antiemetic, *Cannabis Sativa*

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Introduction

Emesis, also known as vomiting, is usually a distasteful condition that results in the forcible ejection of stomach objects through the mouth and is distinctly connected with gastrointestinal motor activity. Therefore, vomiting can be interpreted as a protective response of the body to certain drugs, disease comorbidities, or food toxins [1]. Emesis can occur for various reasons, including illnesses such as food poisoning, motion sickness, gastroenteritis (diarrhea), intestinal obstruction, head injury, pregnancy, appendicitis, or hangover. In addition, vomiting also occurs as a side effect of various exposures: brain tumors, overexposure to ionizing radiation, high intracranial pressure cancer chemotherapy, and radiation therapy [2, 3]. Mechanisms of vomiting in the human body are quite complex. The brain's vomiting center (VC) plays a significant role in triggering vomiting or nausea. The VC is located in an area of the human brain called the chemoreceptor trigger zone (CTZ) of the fourth ventricle [4]. In addition to the CTZ, some other regions, such as the gastrointestinal (GI) tract, higher centers in the cortex, vestibular system, and the thalamus, have also been reported in the literature to trigger vomiting [5]. Vomiting is triggered when the proteins in the VC system are stimulated. During vomiting, the stomach muscle relaxes, and Hydrochloric acid THC(HCl) secretion is prevented. Intensive backward contraction of the small intestine stimulates the stomach to cause retching and vomiting, and vomiting occurs [6]. Today, most common drugs used to treat nausea and vomiting are serotonin (5-HT3A) antagonists [7].

The search for new antiemetic drugs derived from natural sources continues. Flavonoids, cannabinoids, chalcones, glycosides, hydroxycinnamic acids, diarylheptanoids, lignans, phenylpropanoids, saponins, polysaccharides, and terpenes as new antiemetic drug candidates are some of the preferred bioactive chemicals in drug research [8].

Cannabis sativa (C.Sativa) has been used by people for over 5000 years in many fields, for example, the food, textile, and pharmaceutical industries. According to current research, the cannabis plant has been associated with more than one pharmacological activity: relieves chronic pain and muscle spasms, reduces nausea during chemotherapy, increases appetite in human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) patients, improves sleep, and reduce tics in Tourette syndrome [9, 10]. The most active class of compounds in the cannabis plant are phytocannabinoids.

Cannabinoids include compounds such as Δ^9-tetrahydrocannabinolic acid (Δ^9-THC), the primary psychoactive compound, and non-psychoactive cannabidiolic acid (CBDA).

The primary cannabinoids in the plant exist in an acidic form, but due to aging, heat, and exposure to ultraviolet (UV) light, Tetrahydrocannabinolic acid (THCA), the acidic precursor of both CBDA and Δ^9-THC, gradually loses its carboxyl group over time, and cannabidiol (CBD) and Δ^9-THC are formed [10].

There is evidence from studies that Cannabidiol (CBD) is effective in preventing nausea and vomiting. The structure of CBDA and its isolation from cannabis were first described in 1965. Numerous pharmacological effects of CBD have been reported in the literature, but there are few studies on CBDA [11]. Compared to CBD in one study, CBDA was more effective in preventing vomiting in mice and nausea in mice [12]. Consequently, CBDA is promising as a treatment for nausea and vomiting, including anticipatory nausea, for which no specific therapy is available [12].

Screening for the 5HT3A receptor and comparing its affinities with known activators/inhibitors can aid in scoring and evaluating the efficacy of cannabidiolic acid. This study aims to determine the therapeutic effect of CBDA on nausea and vomiting using in silico methods.

Material and Methods

Determination of pharmacological properties of the compound by parameter

SwissADME, a free access web tool used to help estimate ADME. The SMILES form of the CBDA molecule was taken from PubChem and entered into the SwissADME web tool, and the results were obtained.

Target protein and ligand preparation

The three-dimensional structure of the 5HT3A target proteins was taken from the RCSB PDB. 5-HT3A crystallographic structures with PDB identity 6Y5A (Resolution: 2.80 Å) were used. BIOVIA Discovery Studio 2021 program, the water molecules in the protein were deleted from the structure, and the bound ligand residues were removed from the structure. Ligands and other atoms, missing polar hydrogens, were added. Energy minimization was done to achieve stable conformation. Selected ligand (CBDA) structure PubChem (PubChem ID: 160570) was obtained from PubChem chemical compounds database (accessed 17.06.2023). The protein's 3D structure and polarization image were obtained using the PyMOL tool.

Molecular docking

In the study, the AutoDock Vina tool (version 1.5.7) was used to investigate the molecular interaction between target proteins and the selected ligand. Before docking analysis, using the BIOVIA Discovery Studio 2021 program the structure of the enzyme was optimized by removing excess ligands and water molecules. Then all compounds were optimized for energy using the Spartan 14 (Version 1.1.4) program. Polar hydrogens were added to the protein using the AutoDock vina 1.5.7 tool, and Kollman charges were determined as partial charge of compounds calculated using Compute Gasteiger. BIOVIA Discovery was used to determine the active sites of proteins. The x, y, and z coordinates were determined to bind the proteins to the catalytic site. After protein and ligand preparation in Autodock Vina, a grid box was generated. Center points of the box were X =147.3650; Y = 128.7920; Z = 162.5790 and dimensions were X = 90; Y = 90; Z = 90 (all are in Angstrom). Finally, molecular interactions and binding types between the selected compound and target protein were investigated using the Discovery Studio and Ligplot (version 2.2.8) visualizer programs [13].

Results

3D Prediction of target protein

To better understand the 3D structure of the target protein, the 3D structure and surface polarization image of the target protein were obtained using the PyMOL tool. Surface

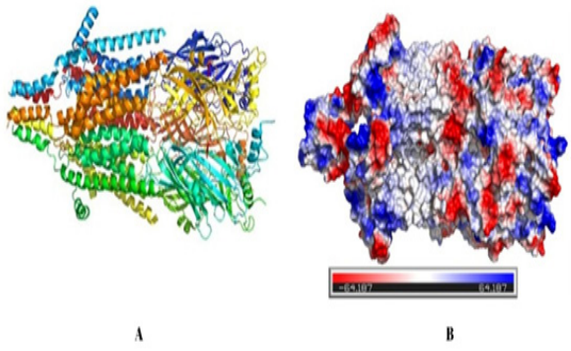

Figure 1. 3D structure (A) and polarization (B) image of 5HT3A protein.

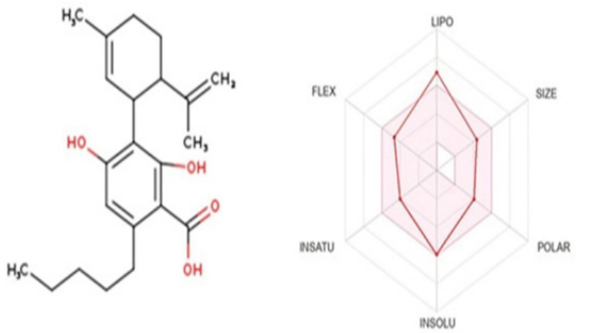

Figure 2. Chemical 2D structure and bioavailability radar of CBDA.

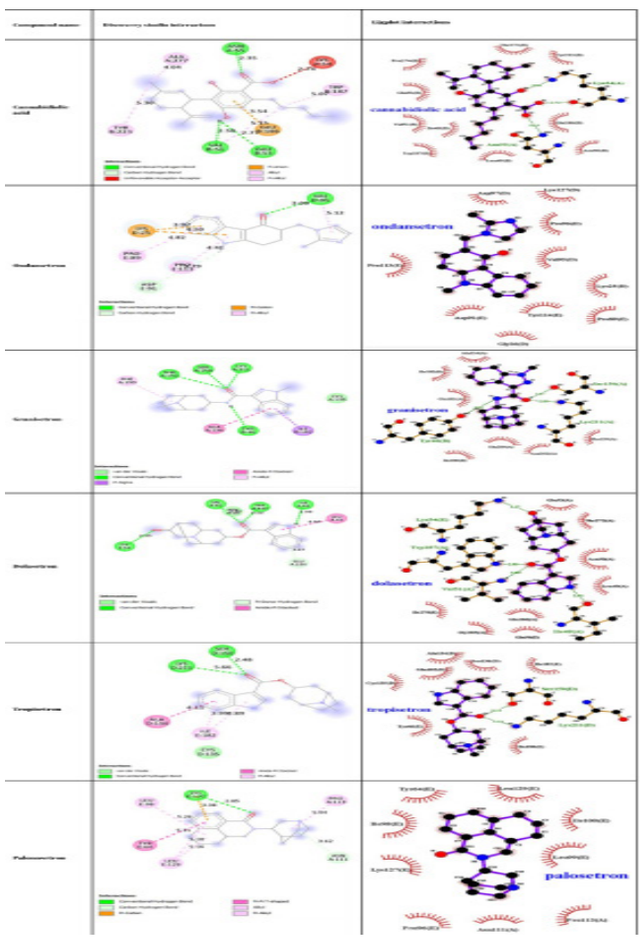

Figure 3. Interactions of ligands with target proteins.

Table 1. Pharmacokinetic properties of CBDA using the SwissADME web server.

Properties	Parameters	CBDA
Physicochemical Properties	Formula	C22H30O4
	MW	358,47 gr/mol
	num. H bond acceptors	7
	num. H bond donors	4
Lipophilicity	iLOGP	3.45
	XLOGP3	6.60
	MLOGP	3.79
Water Solubility	Log S (ESOL)	-5.93
	Class	moderately soluble
Pharmacokinetics	GI	high
	BBB	No
	LogKp	- 3.80 cm/s
	p- gp substrate	No.
	CYP1A2 inhibitor	No.
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	yes
	CYP2D6 inhibitor	No.
	CYP3A4 inhibitor	yes
Drug-likeness	Lipinski	yes; 0 violations

Table 2. Binding affinity value, hydrogen, hydrophobic and other interactions of 5HT3A (6Y5A) receptor with CBDA and reference drugs.

	Compound name	Binding affinity (Kcal/mol)	Hydrogen bonds	Hydrophobic interactions	Others
1.	Cannabidiolic acid	-7.0	VAL51(2.50Å) GLU53(2.77Å) ASN55(2.35Å)	ALA277(4.04Å) TRP187(5.07Å) TYR223(5.30Å)	LYS54(2.74Å) GLU186(3.54Å)
2.	Ondansetron	-7.5	VAL95(3.00Å) ASP91(3.79 Å)	PRO89(4.82 Å) PRO113(4.41 Å)	LYS25(3.97Å,4.50Å)
3.	Granisetron	-8.2	THR152(2.87Å) SER150(2.48Å) LYS211(2.25Å) TYR46(2.28Å)	PHE199(4.91 Å) ALA134(4.45 Å) ILE182(4.34 Å)	-
4.	Dolasetron	-8.5	LYS54(2.55Å) VAL51(2.49Å) TRP187(2.12Å) ILE48(1.99Å) GLU186(3.07Å)	LEU49(4.00 Å)	ASN50 (WAN DER WALS)
5.	Tropisetron	-7.0	SER150(2.48Å) LYS211(5.66Å)	ALA134(4.11Å) ILE182(3.99Å,4.89Å)	-
6.	Palonosetron	-7.9	LYS127(2.85Å) ASN111(3.62Å)	PRO113(5.04 Å) LEU99(5.26 Å) TYR64(5.21 Å) LEU129(5.16Å,5.10 Å)	LYS127(3.38 Å)

polarization shows the charges on the protein surface. Parts shown in blue demonstrate positive charges, parts shown in red have negative charges, and parts seen in white have neutral charges. It can be seen that negative and positive charges are equally distributed on the surface of the 5HT3A protein (Figure 1).

Pharmacological properties of CBDA

Bioavailability radar results, including the chemical structure of the molecule and lipophilicity, size, polarity, solubility, saturation, and flexibility properties, were found on the SwissADME web server (Figure 2).

In addition to these results, in the water solubility parameter, according to the Estimated Solubility (ESOL) filter, CBDA is moderately soluble, GI is high, blood-brain barrier (BBB) is not permeable, there is no substrate for Pg proteins, it is an inhibitor of CYP2C9 and CYP3A4 enzymes, and it complies with the Lipinski rules in drug similarity, and there is no violation (Table 1).

Molecular-docking study of the inhibition of 5HT3A by CBDA

The hydrogen, hydrophobic, and other interactions between protein and ligands as a result of the coupling study with the 5HT3 receptor with cannabidiolic acid and reference drugs are shown in Table 2. Reference drugs and interactions of CBDA with 5HT3 are shown in Figure 3.

The docking study with CBDA and 5HT3A showed a-7.0 kcal/mol binding affinity value. This value is the same as the reference drug tropisetron. It is also very close to the binding affinity values of other reference drugs. CBDA has formed very strong hydrogen bonds with 5HT3A, Val51 (2.50Å), Glu53 (2.77Å), Asn55 (2.35Å) amino acids. Close distance between hydrogen bonds indicates strong hydrogen interactions. Dolasetron, one of the reference drugs, showed a common Val51 hydrogen interaction with CBDA. At the same time, the hydrogen interaction of dolasetron showed a hydrophobic interaction between Trp187 amino acid and cannabidiolic acid. Dolasetron shows an electrostatic interaction with Glu186 and hydrogen bonding with CBDA. In addition, Ala277 (4.04Å) and Tyr223 (5.30Å) showed hydrophobic and Lys54 (2.74Å) acceptor-acceptor interactions. Compared with other reference drugs, dolasetron showed the best interaction at-8.5 kcal/mol, and CBDA showed the most common amino acid interactions with dolasetron.

Discussion

Life arose as a result of interactions of biomolecules at the nanoscale. With the understanding of biomolecular interactions at the nano (atomistic) level, comprehensive information can be obtained from what goes wrong when a disease occurs, to which drugs should be developed to treat that disease. This understanding paved the way for the emergence and rapid development of the structural biology department. In recent years, the field of structural biology cannot keep up with the data generation rate of genetics, biochemistry or various 'omics' sciences, which have grown faster than it. Computational structural biology techniques have emerged to close the production rate gap between all these disciplines. Thanks to these techniques, it has been possible to quickly and reliably predict and describe the atomistic level structures of biomolecules and their interactions in the computer environment. Molecular docking allows us to examine the interactions between receptors and target ligands underlying diseases, so that the most suitable ligand for the receptor can be selected.

The SwissADME, a free web tool used in evaluating the pharmacokinetics, drug-likeness (physicochemical and ADME properties) and medicinal chemistry friendliness of small molecules [14], would be used in testing the drug-likeness of the CBDA. The physicochemical properties of the CBDA were checked using the online tool for their adaptability with Lipinski's rule of five. Lipinski and co-workers proposed the "Rule of Five" in 1997, which was the original and most known rule-based filter for drug-likeness of a molecule, distinguishing whether a molecule can be orally absorbed well or not, following the criteria: molecular weight (MW) ≤ 500, octanol/water partition coefficient (AlogP) ≤ 5, number of hydrogen bond donors (HBD's) ≤ 5 and number of hydrogen bond acceptors (HBAs) ≤ 10.6. According to the Rule of Five, a molecule would not be orally active if it violates two or more of the four rules [15].

CBDA were assessed for their drug-likeliness (ADME and physicochemical properties). CBDA has not violated the Lipinski rule of five, a prominent principle used in certifying the drug-likeness of a compound and this shows that it has passed the drug-likeness test as shown in Table 1. Figure 2 shows the bioavailability RADAR. The bioavailability RADAR enables a first glance at the drug-likeness of a molecule. The pink area signifies the ideal range for each property (lipophilicity: XLOGP3 between −0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å2, solubility: log S not higher than 6, saturation: fraction of carbons in the sp3 hybridization not less than 0.25 and flexibility: no more than 9 rotatable bonds) and GI absorption was also high [14].

Discovery Studio and Ligplot visualizer programs show us the interactions between the receptor-ligand at the amino acid level. Thus, the interactions of the receptor with the active site can be examined. Based on these results, the effectiveness of the reference drugs and the ligand can be compared.

The CBDA insertion study showed a binding affinity value of -7.0 kcal/mol. As a reference, the drugs showed a value close to the binding affinity of CBDA. The fact that the binding affinity values are close to each other indicates that the CBDA molecule can be a drug. It can be seen that CBDA forms very strong hydrogen bonds with the amino acids Val51 (2.50Å), Glu53 (2.77Å), and Asn55 (2.35Å) belonging to 5HT3A. In addition, close distance between the hydrogen bonds indicates that the hydrogen interactions are strong and that CBDA is well located in the protein's active site. This shows that it has an inhibitory effect on 5HT3A. CBDA, Ala277 (4.04Å) and Tyr223 (5.30Å) hydrophobic and Lys54 (2.74Å) acceptor-acceptor interactions were activated. Hydrophobic interactions increase the likelihood of binding to other amino acids so that CBDA can exert a better inhibitory effect on the protein. Dolasetron showed the best binding affinity among the reference drugs but also showed the most common amino acid interactions with CBDA. This shows that cannabidiolic acid can inhibit protein and be a candidate for antiemetic drugs with its strong interactions with 5HT3A

and better binding affinity.

These results show that it can be an inhibitor as effective as dolasetron, which is used as a drug today. When the inhibitory effects of ondansetron, granisetron, tropisetron, palosetron, dolasetron and CBDA, which are frequently used for antiemetic treatment today, are compared, CBDA interacted with more amino acids than all other reference drugs. All these results mean that CBDA can be an inhibitor that is at least as effective as the reference drugs used.

Conclusion

Considering the results of our study, it is seen that CBDA complies with Lipinski's rules with its pharmacokinetic properties. According to our docking study, it has been examined that it has good binding energy and establishes strong amino acid interactions with the target protein. With these properties, it has been seen that it can be a therapeutic candidate for nausea and vomiting.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Conflict of interest

The authors declare no conflict of interest.

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